CYP2E1 and *NQO1* genotypes, smoking and bladder cancer

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Background **Cytochrome P450 2E1 (CYP2E1) and NAD(P)H:quinone oxidoreductase (NQO1) catalyze the activation of some environmental procarcinogens present in tobacco smoke (i.e. nitrosoamines and heterocyclic amines). We conducted a hospital based case–control study to evaluate the potential association between genetic polymorphisms of *CYP2E1* ($C^{1019}T$ in the 5′ flanking region) and *NQO1* ($C^{609}T$ in exon 6) and bladder cancer risk in Asian population.**

Methods **The study population was comprised of 218 histologically confirmed prevalent bladder cancer cases and 199 controls without cancer or systemic illness. PCR–restriction fragment length polymorphism based methods were used for the genotyping analyses and unconditional logistic regression model for the statistical evaluations.**

Results **The risk of bladder cancer increased with the amount of smoking (*P* for trend < 0.01). The frequency of *CYP2E1* c1/c1 genotype was significantly higher in bladder cancer patients (57.9%) than in the controls (47.9%) (OR = 1.8, 95% CI = 1.1–2.9). Similarly, the *NQO1* C/C genotypes were significantly more prevalent in the patients (45.8%) than in the controls (37.6%) (OR = 1.6, 95% CI = 1.0–2.7). The risk for bladder cancer increased with the number of the putative risk genotypes (*P* for trend = 0.03); the most remarkable risk was observed for heavy smokers with both *CYP2E1* c1/c1 and *NQO1* C/C genotypes (OR = 13.8, 95% CI = 3.9–48.6) when compared to non/light smokers with other genotypes.**

Conclusion **Our findings suggest that *CYP2E1* and *NQO1* genotypes may play an important role in development of smoking related bladder cancer among Korean men.**
Pharmacogenetics **13:349–355 © 2003 Lippincott Williams & Wilkins**

Pharmacogenetics 2003, **13**:349–355

Keywords: *CYP2E1*, *NQO1*, combined effect, genetic polymorphisms, bladder cancer

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Received 7 February 2003
Accepted 26 March 2003

Introduction

Bladder cancer ranks fourth in incidence of cancer in the United States [1]. Its incidence among non-Hispanic white men in US was 33.1/100 000 in 1988–1992 [1], which is similar to that found in most European countries [2]. In Korean men, however, the incidence of bladder cancer appears to be much lower, being 7.8/100 000 in 1989 [3]. These differences may be explained by environmental or by genetic backgrounds. A large difference also exists between sexes in both countries; this malignancy is three to four times more frequent in men than in women [1,3].

Exposure to chemicals in tobacco smoke and other environmental and occupational chemicals has been described as risk factors for bladder cancer in numerous epidemiological and laboratory studies (e.g. 2-naphthylamine, benzidine, 4-aminobiphenyl) [1,4]. These carcinogens are either activated or detoxified by xenobiotic metabolizing enzymes. The inherited differences in the capacity to metabolize these chemicals have been recently suggested as modifiers of individual susceptibility to environmentally-induced bladder cancer.

Cytochrome P450 2E1 (CYP2E1) catalyzes the metabolic activation of various tobacco-related *N*-nitrosamines, such as *N*-nitroso-dimethylamine and *N*-nitrosonornicotine [5], which are potent bladder carcinogens in experimental animals [6]. To date several polymorphisms of the human *CYP2E1* have been identified [7]. One polymorphism site recognized by *Rsa*I digestion in the 5′ flanking region of the gene reported to be associated with decreased enzyme activity or non-inducibility [8,9]. The results of previous epidemiological studies on the associa-

0960-314X © 2003 Lippincott Williams & Wilkins

DOI: 10.1097/01.fpc.0000054096.48725.25

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tion between susceptibility to bladder cancer and this genetic polymorphism have given inconsistent results [10–12]. Moreover, wide interethnic differences have been observed in the frequency of the *CYP2E1* alleles; the variant c2 alleles of this gene are rare in Caucasians but common in Asians [13].

NAD(P)H:quinine oxidoreductase (NQO1) acts as either a detoxification or activation enzyme, depending on the substrate. NQO1 catalyzes the two-electron reduction of quinoid compounds to hydroquinones, readily conjugated to other molecules (e.g. glucuronides) which are subsequently more readily excreted, preventing the generation of free radicals and reactive oxygen, thus protecting cells from oxidative damage [14]. NQO1, however, also catalyzes the activation of some environmental procarcinogens present in tobacco smoke, such as nitrosamines and heterocyclic amines [15,16]. Traver *et al*. demonstrated that C to T transversion at position 609 of *NQO1* cDNA, leading to non-synonymous mutation ($Pro^{187}Ser$), reduced enzyme activity [17]. Recently, Siegel *et al*. reported that no NQO1 protein activity was detected in *NQO1* T/T genotype and low to intermediate activity was detected in *NQO1* C/T genotype compared with C/C genotype [18]. Thus, this polymorphism may result in altered metabolic activation of procarcinogens in tobacco smoke.

The results of previous studies between *NQO1* C/C genotype and lung cancer were inconsistent; mostly increased the risk of lung cancer with C/C [19–22], but not all [23,34]. The association between *NQO1* genotypes and urothelial tumors [25] and leukemia [26] were increased.

We previously reported significant interactive effects between *GSTM1* and *T1* genetic polymorphisms and bladder cancer [27]. Here we extended the study to examine the potential role of *CYP2E1* and *NQO1* genotypes in development of bladder cancer in Korean males. The gene–environment interaction between known risk factors of bladder cancer and genotypes was also assessed.

Methods

Study subjects and selection

The study population consisted of 241 histologically confirmed male bladder cancer cases admitted for treatment to urology departments of three teaching hospitals in Seoul during February 1997–May 2000 (Seoul National University Hospital, Boramae Hospital, and Samsung Medical Center) and 238 male controls with no present or previous history of cancer or systemic illnesses admitted to the same departments. Roughly 95% of cases diagnosed at these hospitals were asked to participate in the study. About 10% of cases and 17% of controls approached were not included in the final study population because of refusal to participate in the study, failure to be interviewed, and/or because no blood samples were available for them.

Among 218 bladder cancer cases for whom information on the number of months from diagnosis to interview was available, the median was 10.7 months. Ninety-two cases had no previous tumor resection. The controls were selected from the same urology departments in the same hospitals using the following criteria: (1) they had no current or previous cancer or systemic disease, and (2) they were males over 40 years old. Thirty-three percent of controls had benign prostate hypertrophy, 20% had kidney or ureter stones, 7% had urethral stricture, and 6% had hydrocele. The remaining 34% were either kidney donors or had other disorders like kidney rupture, obstructive uropathy, scrotal swelling, erection disorder, and penile foreign body.

All study subjects provided informed consent prior to participating in the study. The study was approved by the institutional review board of Seoul National University Hospital. Information on demographic characteristics (education, marital status, weight, height, etc.), usual occupation and history of ever-working at previously identified high-risk industries [1,28], life-style habits including smoking (if they had smoked more than 400 cigarettes in their lifetime, current status of smoking, duration of smoking, amount of cigarettes consumed per day, etc.), alcohol consumption (never, social, heavy, etc.), and history of urinary tract stones and tuberculosis was collected by trained interviewer with a standardized questionnaire.

DNA extraction

Blood was collected in 10-ml heparinized tubes and centrifuged at 3000 r.p.m. for 10 min at room temperature within 10 h of collection. Plasma, buffy coat, and red blood cells were separated and stored at −70°C. The buffy coat (0.5–1 ml) was kept frozen until it was thawed for DNA extraction for this study. DNA was isolated from the buffy coat samples with an Applied QIAGEN extraction kit using protocols and reagents supplied (Chatsworth, CA, USA). The extracted DNA was stored at −20°C until the genotype analyses.

Genotyping analyses

PCR–restriction fragment length polymorphism-based assays were used to characterize the wild-type and variant alleles of *CYP2E1* and *NQO1*. In the *CYP2E1* genotyping analyses primers 5′-CCA GTC GAG TCT ACA TTG TCA-3′ and 5′-TTC ATT CTG TCT TCT AAC TGG-3′ were used in the PCR employing the following amplification conditions: the 20 μl reaction mixtures contained 1.5 mM $MgCl_2$, 250 μM dNTPs, 1 μM primers, 1 μM template DNA and 1 U of Taq

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polymerase with the buffer (10 mM Tris-HCl, pH 9.0; 40 mM KCl; Bioneer, Korea). After an initial denaturation at 94°C for 4 min, 34 cycles of 60 s at 94°C, 60 s at 60°C and 60 s at 72°C were performed, followed by a final extension step of 4 min at 72°C. After PCR an aliquot of the product was digested with *Rsa*I for 3 h at 37°C.

In the *NQO1* analysis the PCR was performed employing otherwise the same amplification conditions as for *CYP2E1* except that primers 5′-TCC TCA GAG TGG CAT TCT GC-3′ and 5′-TCT CCT CAT CCT GTA CCT CT-3′ were used. After an initial denaturation at 94°C for 3 min, 36 cycles of 10 s at 94°C, 30 s at 60°C, and 60 s at 72°C were performed, followed by a final extension step of 5 min at 72°C. After PCR an aliquot of the product was digested with *Hinf*I for 2.5 h at 37°C.

In the genotyping of *CYP2E1*, *Rsa*I digestion produced 360- and 50-bp bands for c1/c1; 410-, 360-, and 50-bp bands for c1/c2; and 410-bp band for c2/c2. In the genotyping of *NQO1*, *Hinf*I digestion produced 195- and 35-bp bands for C/C; 195-, 151- and 35-bp bands for C/T; and 151-, 44- and 35-bp bands for T/T. These digested products were electrophoresed on each 3% metaphor agarose gel for *CYP2E1* and 3% agarose gel for *NQO1* and visualized by ethidium bromide staining.

Statistical analyses

To examine the associations between known or suspected risk factors and bladder cancer, and between the *CYP2E1* and *NQO1* genotypes and bladder cancer risk, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression [29]. The ORs were adjusted for age, urinary tract stone and smoking.

Subjects were divided into 'ever' smokers who had smoked more than 400 cigarettes in their lifetime, and 'never' smokers who had smoked less than 400 cigarettes. When pack-year was defined as the product of [{number of cigarettes consumed per day} × {duration of smoking (years)}], the ever smokers were grouped as 'light' (≤ 25 pack-years) and 'heavy' smokers (> 25 pack-years) by using the mean (25 pack-years) in controls as the cut-off. To increase the statistical power, the respective genotypes were categorized into two groups in the statistical analysis (see Table 2). For evaluation of the gene–dosage and interactive effect, carriers of one or two low activity alleles of *CYP2E1* and *NQO1* were considered as the referents according to previous studies on the genotype–phenotype relationship for these genes [8,18]. Interactive effects between each genotype and smoking status and the linear trend of the gene–dosage effect on bladder cancer risk were evaluated by likelihood ratio test.

Combined genotypes were categorized into three groups according to the numbers of 'at-risk' genotypes of *CYP2E1* (c1/c1 genotype versus c2 allele containing genotypes) and *NQO1* (C/C genotype versus T allele containing genotypes). To test for gene–environment interaction between combined genotypes of *CYP2E1* and *NQO1* and pack-year of smoking, a test for trend in slopes in logistic regression models stratified by the number of at-risk genotypes was used. The gene–environment interaction would manifest itself through different slopes with respect to exposure for different genotypes [30]. Assuming that in the absence of a relevant carcinogenic exposure the metabolic genes play no role in carcinogenesis, all genotypes could use 'simple model' with a common intercept for the test since the intercepts in the model of each genotype were not different ($P = 0.16$) [31].

Results

Selected characteristics of bladder cancer cases and controls are presented in Table 1. The mean age was 63.9 years (SD 9.8 years) and 61.7 years (SD 12.2 years) for bladder cancer patients and controls, respectively. There was a statistically significant dose-dependent increase in risk of bladder cancer associated with smoking (P for trend < 0.01). Although a slightly higher percentage of cases than controls had ever worked in high-risk occupations (29.4 and 27.1%, respectively), no significant differences in the cancer risk were observed between these groups. Previous history of tuberculosis and urinary tract infection was higher in cases than in controls, whereas urinary tract stone was somewhat higher in controls. None of these differences were, however, statistically significant.

The frequencies of *CYP2E1* and *NQO1* genotypes in the controls (Table 2) were similar to those previously observed in Asians [7,13,20,32,33]. The *CYP2E1* c1/c1 genotype posed a significantly increased risk of bladder cancer (OR = 1.8, 95% CI = 1.1–2.9) compared to the c2 allele containing genotypes (c1/c2 or c2/c2); the risk of bladder cancer increased with the number of c1 alleles (P for trend < 0.01). Similarly to the *CYP2E1* c1/c1 genotype the *NQO1* C/C genotype posed an increased risk of bladder cancer (OR = 1.6, 95% CI = 1.0–2.7) compared to the *NQO1* T allele containing genotypes (C/T and T/T), and the OR increased as the number of putative risk genotype increased (P for trend = 0.03; Table 2).

When incident and prevalent cases were examined separately, the distributions of the *CYP2E1* and *NQO1* genotype frequencies were almost the same in both subgroups and no significant differences were seen in these results compared with those for the total case group (data not shown).

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Table 1 Selected characteristics for 218 bladder cancer cases and 199 control subjects

Characteristics	Cases *n* (%)		Controls *n* (%)		*P*-value/OR (95% CI)
Age					
Under 49	22	10.1	36	18.1	
50–59	41	18.8	48	24.1	
60–69	93	42.7	62	31.2	< 0.01 (chi square)
70–79	54	24.8	38	19.1	
Over 80	8	3.7	15	7.5	
Mean age (SD)	63.9	(9.8)	61.7	(12.2)	0.04 (*t*-test)
Smoking					
Non-smokers	31	14.2	37	21.6	1.0
1–25 pack-years	58	26.6	84	49.1	0.9 (0.5–1.6)
> 25 pack-years*	101	46.3	50	29.2	2.7 (1.4–4.9)
Mean pack-years (SD)	28.4	(24.4)	19.8	(20.6)	< 0.01 (*t*-test)
Cigarettes per day (SD)	16.3	(11.9)	12.6	(11.6)	< 0.01 (*t*-test)
Missing	28	12.8	19	9.5	
High-risk occupation**					
No	129	59.2	125	62.8	
Yes	64	29.4	54	27.1	0.74 (chi square)
Missing	25	11.5	20	10.1	
Tuberculosis infection	25	13.3	18	10.1	0.43 (chi square)
Urinary tract infection	20	10.8	14	7.9	0.44 (chi square)
Urinary tract stone	22	11.8	33	18.8	0.09 (chi square)

ORs adjusted for age and urinary tract stone.
*P for trend < 0.01.
**High-risk occupation was defined as positive history of ever working at the presumed high-risk industries (dye, rubber, leather, aluminum, paint, tire manufacturing, petrochemical industry, etc.).

Table 2 Association between *CYP2E1* and *NQO1* genotype and bladder cancer

	Cases *n* (%)	Controls *n* (%)	OR (95% CI)	OR (95% CI)
CYP2E1				
c2/c2	4 (1.9)	12 (6.2)	1.0 (reference)	1.0 (reference)
c1/c2	86 (40.2)	89 (45.9)	2.5 (0.6–10.4)	
c1/c1	124 (57.9)	93 (47.9)	4.3 (1.1–17.4) *P* for trend < 0.01	1.8 (1.1–2.9)
NQO1				
T/T	28 (15.8)	16 (9.4)	1.0 (reference)	1.0 (reference)
C/T	68 (38.4)	90 (52.9)	0.3 (0.1–0.7)	
C/C	81 (45.8)	64 (37.6)	0.7 (0.3–1.5)	1.6 (1.0–2.7)
Combined genotype*				
No risk genotype	39 (22.2)	57 (34.1)	1.0 (reference)	
One risk genotype	89 (50.6)	76 (45.5)	2.1 (1.2–3.9)	
Two risk genotypes	48 (27.3)	34 (20.4)	2.8 (1.4–5.5) *P* for trend = 0.03	

ORs adjusted for age, urinary tract stone and smoking.
*No risk genotype: all *CYP2E1* c2 allele and *NQO1* T allele containing genotypes; one risk genotype: *CYP2E1* c1/c1 genotype or *NQO1* C/C genotype; two risk genotypes: *CYP2E1* c1/c1 genotype and *NQO1* C/C genotype.

Heavy smokers (more than 25 pack-year) with the *CYP2E1* c1/c1 genotype appeared to be at 5.6-fold risk of bladder cancer (95% CI = 2.8–11.2) compared to non/light smokers with the c2 allele containing genotypes. However, the multiplicative interactive effect of smoking and the genotype was not statistically significant (P for interaction = 0.92; Table 3).

Similarly, heavy smokers with the *NQO1* C/C genotype were at 5.4-fold risk (95% CI = 2.5–12.1) compared to non/light smokers with the T allele containing genotypes. Again, the multiplicative interactive effect of smoking and the genotype was not significant (P for interaction = 0.21; Table 4).

The gene–dosage effect of the combined *CYP2E1* and *NQO1* genotypes were evaluated stratified by smoking status (Table 5). The most remarkable risk of bladder cancer (OR = 13.8, 95% CI = 3.9–48.6) was observed for heavy smokers with concurrent presence of *CYP2E1* c1/c1 and *NQO1* C/C genotypes compared to non/light smokers with combination of the *CYP2E1* c2 and *NQO1*

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Table 3 The ORs and 95% CIs for interaction between *CYP2E1* genotype and smoking and bladder cancer

Smoking status	c1/c2 & c2/c2 (cases/controls)	c1/c1 (cases/controls)
Non and light smokers	1.0 (reference) (33/58)	1.8 (1.0–3.3) (55/61)
Heavy smokers	3.2 (1.6–6.2) (44/27)	5.6 (2.8–11.2)* (54/21)

ORs adjusted for age and urinary tract stone. Non and light smokers ⩽ 25 pack-years; heavy smokers > 25 pack-years.
*P for interaction = 0.92.

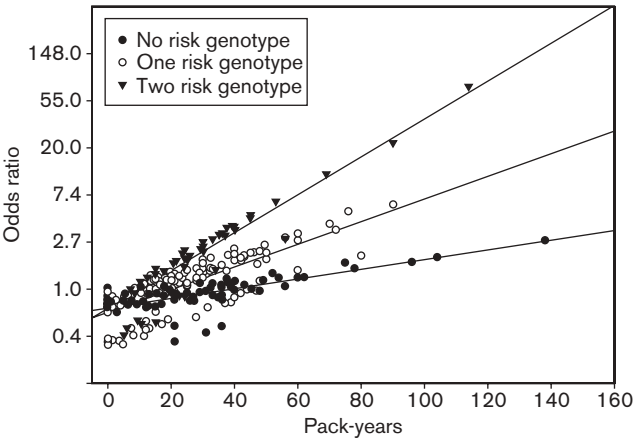
Table 4 The ORs and 95% CIs for interaction between *NQO1* genotype and smoking and bladder cancer

Smoking status	T/T & C/T (cases/controls)	C/C (cases/controls)
Non and light smokers (⩽ 25 pack-year)	1.0 (reference) (37/59)	1.3 (0.7–2.4) (35/41)
Heavy smokers (> 25 pack-year)	2.2 (1.2–4.1) (46/32)	5.4 (2.5–12.1)* (40/13)

ORs adjusted for age and urinary tract stone.
*P for interaction = 0.21.

T alleles containing genotypes (Table 5; P for trend in slope across genotypes = 0.01, Fig. 1).

Fig. 1



Relationship between bladder cancer risk and pack-year stratified by the combined genotypes of *CYP2E1* and *NQO1*. (P for trend in slopes across genotypes = 0.01.) No risk genotype: all *CYP2E1* c2 allele and *NQO1* T allele containing genotypes; one risk genotype: *CYP2E1* c1/c1 or *NQO1* C/C genotype; two risk genotypes: *CYP2E1* c1/c1 genotype and *NQO1* C/C genotype.

Discussion

In the present study 1.8-fold and 1.6-fold risk of bladder cancer was observed for individuals with *CYP2E1* c1/c1 and *NQO1* C/C genotypes, respectively. Although our findings are supported by recent studies showing that the *CYP2E1* c1/c1 genotype was associated with increased risk of smoking-related cancers such as lung, esophagus, and oral cancer [7,34,35], the previous studies on bladder cancer gave contrasting outcomes. Anwar *et al*. failed to demonstrate a difference in the distribution of *CYP2E1* polymorphism between Egyptian bladder cancer patients and controls, although the study included only 22 bladder cancer cases and 20 controls [11]. On the other hand, in a larger hospital based case–control study with 374 German bladder cancer cases and 383 controls, a similar lack of association was observed [10]; although the c2 allele appeared to be more frequent in controls compared to cases, the overall genotype frequency was not significantly different between these study groups. Moreover, Farker *et al*. also conducted a German study on *CYP2E1* genetic polymorphism in renal cell/urothelial cancer patients and observed no significant difference in the genotype frequencies between 224 renal cell/urothelial cancer patients and 304 controls [12].

Table 5 The ORs and 95% CIs for interaction between the combined genotype of *CYP2E1* and *NQO1* and smoking and bladder cancer

	Non and light smokers (cases/controls)	Heavy smokers (cases/controls)
No risk genotype	1.0 (reference) (13/31)	2.9 (1.2–7.4) (22/18)
One risk genotype	2.2 (1.0–5.1) (37/43)	5.4 (2.3–13.1) (40/19)
Two risk genotypes	2.2 (0.9–5.5) (22/25)	13.8 (3.9–48.6) (23/6)
P for trend	0.10	0.03

ORs adjusted for age and urinary tract stone. Non and light smokers ⩽ 25 pack-years; heavy smokers > 25 pack-years.
No risk genotype: all *CYP2E1* c2 allele and *NQO1* T allele containing genotypes; one risk genotype: *CYP2E1* c1/c1 genotype or *NQO1* C/C genotype; two risk genotypes: *CYP2E1* c1/c1 genotype and *NQO1* C/C genotype.

Similarly to *CYP2E1* genotype data, the findings of the significant association between *NQO1* genotypes and bladder cancer are supported by the previous studies on the association between *NQO1* genotypes and lung cancer conducted in Asian population [20–22,36]; however, these findings are not consistent with the results from the studies of Caucasians (23,24). The studies on bladder cancer have given inconsistent results. Schulz *et al*. investigated the distribution of *NQO1* genotype in urothelial carcinoma patients ($n = 99$) and a normal population ($n = 260$) [25]. Although 3.6-fold increased frequency of the *NQO1* T/T genotypes were observed in the tumor patients compared to controls, the prevalence of T/T genotype was very small in both groups (4 in cases; 5 in controls), and the confidential intervals were wide (OR = 3.6, 95% CI = 0.9–13.7). In the case–control study by Peluso *et al*. no significant difference in distribution of *NQO1* genotype frequency was observed between 123 cases and 54 controls [37].

The possible explanations for the above inconsistent results are interethnic difference in allele frequency of the gene. There are differences in frequencies of the

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genes between Asian and Caucasian [13,38]. The frequency of *CYP2E1* c2 allele (29%) in the present study is similar to that previously reported in Asian (24–30%) [7,13,32,33], but much higher than that in Caucasian (2–3%) [13,35], African-American (0.3–7%) [13,34,35] and Mexican-American (15%) [34]. The frequency of *NQO1* T allele (37%) in this study is also similar to that previously founded in Asian (38–42%) [19–23,38] and Mexican-American (40%) [19,38], but much higher than that in Caucasian (11–19%) [23–25] and African-American (22%) [19,38]. Higher frequencies of variant alleles observed in this study might increase the statistical power in limited sample size.

When the combined genotype effects were examined, concurrent presence of at-risk genotypes of both *CYP2E1* and *NQO1* posed more than a 13-fold risk of bladder cancer in heavy smokers compared to non/light smokers with the other genotype combinations. Moreover, there was a significant trend in bladder cancer risk with pack-year of smoking, which followed the number of at-risk genotypes (Fig. 1). This is suggestive of a synergistic function between *CYP2E1* and *NQO1* genotypes and cigarette smoking in the etiology of bladder cancer.

The potential functional basis for the observed effect of *CYP2E1* genotypes in the bladder cancer risk may be in that tobacco-related nitrosamines are activated primarily by CYP2E1 [5] and these compounds can induce tumors of various types of cancer including bladder cancer. Dibutylnitrosamine is a bladder carcinogen and is metabolized in the liver by CYP2E1 to give *N*-4-hydroxybutyl-*N*-butylnitrosamine, which is oxidized to *N*-3-carboxypropyl-*N*-butylnitrosamine [6] and then excreted in the urine and absorbed and activated in the bladder mucosa [39,40].

NQO1, on the other hand, is also involved in metabolic activation of heterocyclic amines present in cigarette smoke, 2-amino-3-methylimidazo[4, 5-f]quinoline and 3-amino-1-methyl-5H-pyrido[4, 3-b]indole [41,42], which induced bladder carcinoma in animals [14,43]. Bergamaschi and coworkers reported that an increased reaction rate between O_3 and hydroquinones would be consistent with the greater increase in 8-hydroxy-2′-deoxyguanosine after O_3 exposure in the subjects carrying both the *NQO1* C/C and *GSTM1* null genotype compared to people with other genotypes; this suggested that individuals with combination of *NQO1* and *GSTM1* at-risk genotypes may be more susceptible for oxidative stress [44]. Recently, Choudry *et al*. found the elevated NQO1 levels in human bladder tumor tissue exist in a subset of patients [45].

There are also some limitations in this study like that of the statistical power to analyze the association between the genotypes and bladder cancer was limited by modest sample size and that the controls were recruited from hospital patients. The main limitation was that the cases included patients diagnosed more than 12 months prior to the study and/or patients with recurrent tumor development. Therefore, although the genotype frequencies were not significantly different across case subgroups, a larger study of newly diagnosed primarily cases in the same populations are needed to verify the present findings.

In conclusion, our findings suggest that the *CYP2E1* and *NQO1* genotypes may play important role in development of bladder cancer among Korean men and that the association between cigarette smoking and bladder cancer may be modulated by these genetic polymorphisms.

Acknowledgement

This work was supported by a Korea Research Foundation Grant, 1998 (97110784) and in part by 2000 BK21 project for Medicine, Dentistry and Pharmacy.

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